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(57) Abstract

The present invention provides *cis* element–reporter constructs for measuring transcription. A series of *cis* elements are linked to a reporter gene to generate *cis*–acting reporters. Examples of reporter genes are secreted alkaline phosphatase (SEAP), destabilized green fluorescent protein (d2EGFP) and luciferase. Also provided are various applications of such constructs.

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CIS-ELEMENT REPORTER CONSTRUCTS AND USES THEREOF

BACKGROUND OF THE INVENTION

Cross-Reference to Related Applications

This application claims benefit of priority of pending non-provisional application U.S. Serial No. 09/206,887, filed December 8, 1998.

Field of the Invention

The present invention relates generally to the field of molecular biology of transcription. More specifically, the present invention relates to *cis* element reporter constructs for measuring transcription.

Description of the Related Art

Regulation of the activity of transcription factors is believed to be one of the most important biochemical controls to signal transduction, cell proliferation, differentiation, apoptosis, development, and disease. Studying the regulation of an individual transcription factor in various specific biological functional system has yielded a tremendous amount of information; however, the information that one can obtain from the current strategies of studying an individual factor is limited, despite the global functional status of a number of transcription factors. The information is limited because cross-talking among the different signal transduction pathways leads to activation of multiple transcription factors. Therefore, there is a need

to provide a tool to study the interaction and overall effect of various transcription factors.

Green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* is a reporter molecule for monitoring gene expression and protein localization *in vivo*, *in situ* and in real time (1-4). GFP fluoresces bright green upon mere exposure to UV or blue light-unlike other bioluminescent reporters which require additional proteins, substrates, or cofactors to emit light. GFP fluorescence is stable, species independent and can be monitored noninvasively in living cells. GFP fluorescence persists in formaldehyde-fixed cells and is well suited for double-labeling experiments with other fluorescent markers, including the GFP variant enhanced green fluorescent protein (EGFP, GFP_{mut1}; 5-6).

EGFP encodes a protein which has a single, red-shifted excitation peak and fluoresces about 35 times more intensively than wild type GFP when excited at 488 nm (7), due to an increase in its extinction coefficient (Em). To ensure maximal mammalian expression, the coding region of EGFP contains more than 190 silent base mutations which correspond to human codon-usage preferences (7). The red-shifted spectrum and increased expression of EGFP make it ideal for fluorescence microscopy and fluorescence-activated cell sorting (FACS; 5, 8).

Destabilized EGFP (d2EGFP) contains the PEST domain from mouse ornithine decarboxylase (MODC), fused to the C-terminus of EGFP (9). This domain targets EGFP for rapid turnover effectively reducing EGFP's half-life to two hours. The introduction of d2EGFP greatly increases the utility of GFP in studying dynamic cellular-events *in vivo*.

Another reporter molecule, secreted alkaline phosphatase (SEAP, 10-11), is a secreted form of human placental alkaline

phosphatase and has been used as a reporter for the analysis of *cis*-acting DNA sequences and *trans*-acting factors. Secreted alkaline phosphatase is efficiently secreted from cells, and the levels of SEAP activity in the culture medium are directly proportional to changes in intracellular concentrations of SEAP mRNA and protein (10, 12). Secreted alkaline phosphatase differs from native placental alkaline phosphatase (AP) only in the absence of a membrane-anchoring domain. The secreted nature of secreted alkaline phosphatase offers many advantages over other transcription reporter molecules. Most notably, it is not necessary to prepare cell lysates to assay for the reporter protein. Because the transfected cells remain intact, the kinetics of gene expression can be studied easily using the same cultures by sampling the medium repeatedly; therefore, set-up and variability for multiple cultures are essentially eliminated.

Another important advantage of secreted alkaline phosphatase as a transcription reporter is that background signals due to endogenous alkaline phosphatases are nearly absent. Unlike most endogenous alkaline phosphatases, secreted alkaline phosphatase is extremely heat-stable and resistant to the inhibitor L-homoarginine. Therefore, endogenous alkaline phosphatase activity can be eliminated effectively by preheating the culture medium sample as 65°C and assaying in the presence of L-homoarginine.

In addition, luciferase is a widely used reporter. It is one of most sensitive reporters and like GFP and SEAP, may be fused to a transcription factor of interest and used to track the activation of the transcription factor of interest.

The prior art is deficient in the lack of effective means of detecting and measuring the activities of a number of transcription factors. Further, the prior art is deficient in the lack of a construct for

measuring transcription. The present invention fulfills this long-standing need and desire in the art.

SUMMARY OF THE INVENTION

The present invention is directed to novel cis-acting reporters for measuring transcription. In one embodiment of the present invention, there is provided a *cis* element-reporter construct comprising a *cis* element, a reporter gene and a promoter. Representative *cis* elements include nuclear factor-κB, heat responsive element, Myc, p53, nuclear factor of activated T cells, activator protein 1 (AP1), serum responsive element, glucocorticoid responsive element and cAMP responsive element. Representative reporter genes include secreted alkaline phosphatase (SEAP), destabilized green fluorescent protein (d2EGFP) and luciferase (Luc). Representative promoters include thymidine kinase promoter and gonadotropin α-gene promoter.

In another embodiment of the present invention, there is provided a method of generating a *cis* element-reporter construct by linking a *cis* element to a promoter and then fusing the element to a reporter gene.

In yet another embodiment of the present invention, there is provided a method of monitoring activation of a transcription factor, comprising the steps of generating a vector comprising a *cis* element, a reporter gene and a promoter, wherein the *cis* element is a component of a DNA sequence to which said transcription factor binds to ensure activity; transfecting a cell line with the vector; and detecting expression of the reporter gene, wherein expression of the reporter

gene indicates activation of the transcription factor. Representative cell lines include CHO, HEK 293, Saos2 and HeLa.

In still yet another embodiment of the present invention, there is provided a method of determining whether a gene is involved in a signal transduction pathway, comprising the steps of generating a vector comprising a *cis* element, a reporter gene and a promoter, wherein the *cis* element is a component of a DNA sequence which affects the expression of the a gene; expressing the vector in a cell line, wherein the cell line is associated with the signal transduction pathway; and detecting the expression of the reporter gene, wherein expression of the reporter gene indicates the involvement of the gene in the signal transduction pathway.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention briefly summarized above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figure 1 shows the restriction map of pNF-κB-SEAP.

Figure 2 shows the restriction map of pNF-κB-d2EGFP.

Figure 3 shows the restriction map of pNF-κB-Luc.

Figure 4 shows mediated induction of transcription factors in HEK 293 cells. Twenty four hours after transfection, cells were treated with an inducer for 6 hours. Samples were collected and measured for SEAP activity. **Figure 4A** shows TNF-mediated transcriptional induction. Cells were treated with 100 ng/ml TNF; NFκB and NFAT signaling pathways exhibit 4-fold and 10-fold induction, respectively, when activated by TNF. **Figure 4B** shows serum-mediated induction of transcription factors in HEK 293 cells. Cells were fed with 10% serum. Multiple pathways were induced, when activated by serum. In both experiments, pTK-SEAP was used as the negative control. (RLU = relative light units)

DETAILED DESCRIPTION OF THE INVENTION

The following terms have the definitions set below.

As used herein, "*cis*-element" means a genetic element having the ability to affect the activity of a gene on the same DNA molecule as that genetic element.

As used herein, "reporter" means a molecule (usually a protein) that is expressed in response to or as a result of a particular biological or molecular event.

As used herein, "cis-acting reporter" means a protein that is expressed in a measurable manner in response to a molecular event that takes place on the same DNA strand that the DNA which codes for the protein is located.

As used herein, "transcription factor" means any factor that controls genetic transcription.

As used herein, "signal transduction pathway" means the pathway or sequence of molecular events which converts an extracellular signal into cellular activity.

The present invention is directed to a series of *cis* element-reporter constructs and uses thereof.

In one embodiment of the present invention, there is provided a *cis* element-reporter construct comprising a *cis* element, a reporter gene and a promoter. Representative *cis* elements include nuclear factor-κB, heat responsive element, Myc, p53, nuclear factor of activated T cells, activator protein 1, serum responsive element, glucocorticoid responsive element and cAMP responsive element. In one preferred embodiment, the nuclear factor-κB has the sequence shown in SEQ ID No: 1. In one preferred embodiment, the heat responsive element has the sequence shown in SEQ ID No: 2. In one preferred embodiment, the Myc has the sequence shown in SEQ ID No: 3. In one preferred embodiment, the p53 has the sequence shown in SEQ ID No: 4. In one preferred embodiment, the nuclear factor of activated T cells has the sequence shown in SEQ ID No: 5. In one preferred embodiment, the activator protein 1 has the sequence shown in SEQ ID No: 6. In one preferred embodiment, the serum responsive element has the sequence shown in SEQ ID No: 7. In one preferred embodiment, the glucocorticoid responsive element has the sequence shown in SEQ ID No: 8. In one preferred embodiment, the cAMP responsive element has the sequence shown in SEQ ID No: 9 or SEQ ID No:10. Representative reporter genes include secreted alkaline phosphatase (SEAP), destabilized green fluorescent protein (d2EGFP) and luciferase (Luc). Representative promoters include thymidine

kinase promoter, gonadotropin α-gene promoter, and other known by those having ordinary skill in this art.

In another embodiment of the present invention, there is provided a method of generating a *cis* element-reporter construct by linking a *cis* element to a promoter and then fusing the element to a reporter gene.

In yet another embodiment of the present invention, there is provided a method of monitoring activation of a transcription factor, comprising the steps of generating a vector comprising a *cis* element, a reporter gene and a promoter, wherein the *cis* element is a component of a DNA sequence to which said transcription factor binds to ensure activity; transfecting a cell line with the vector; and detecting expression of the reporter gene, wherein expression of the reporter gene indicates activation of the transcription factor. Representative cell lines include CHO, HEK 293, Saos2 and HeLa.

In still yet another embodiment of the present invention, there is provided a method of determining whether a gene is involved in a signal transduction pathway, comprising the steps of generating a vector comprising a *cis* element, a reporter gene and a promoter, wherein the *cis* element is a component of a DNA sequence which affects the expression of a test gene; expressing the vector in a cell line, wherein the cell line is associated with the signal transduction pathway; and detecting the expression of the reporter gene, wherein expression of the reporter gene indicates the involvement of the gene in the signal transduction pathway.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

Cis-Element

The following *cis* elements were utilized for constructing cis-acting reporters:

NF-κB (nuclear factor-κB) having the sequence of GGGAA TTTCCGGGAATTTCCGGGAATTTCCGGGAATTTCC (SEQ ID No: 1);

HRE (heat responsive element) having the sequence of TAGAATGTTCTAGATCTAGAACATTCTAGCTAGAATGTTCTAG (SEQ ID No: 2);

Myc having the sequence of CACGTGCACGTGCACGTGCACG TGCACGTGCACGTG (SEQ ID No: 3);

p53 having the sequence of TCGAGCTTGCCTGGACTTGCCTG GGCCAGATCTGTCGACGGAGGTCGAGCTTGCCTGGACTTGCCTGCCCACATCT GTCGACGGAGGTCGAGCTTGCCTGGACTTGCCTGCCCACATCTGTCGACGGAG GTCGAGCCTTGCCTGGACTTGCCTGCCA (SEQ ID No: 4);

NFAT (nuclear factor of activated T cells) having the sequence of GGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAA CTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCGT (SEQ ID No: 5);

AP1 (activator protein 1) is an active protein and its activity is composed primarily by Jun/Fos heterodimers as well as Jun homodimers. The activity of AP1 responds to growth factors, such as phorbol ester (TPA), protein kinase C. Ap1 having the sequence of TGAGTCAGTGAGTCACTGACTCACTGACTCATGAGTCAGCTG ACTC (SEQ ID No: 6);

SRE (serum responsive element) having the sequence of GATGTCCATATTAGGACATCGATGTCCGAATATGGACATCGATGTCCATATT AGGACATC (SEQ ID No: 7);

GRE (glucocorticoid responsive element) having the sequence of GGTACATTTTGTTCTAGAACAAAATGTACCGGTACATTTTGT TCT (SEQ ID No: 8);

CRE stands for cAMP responsive element. Two CRE elements were utilized: CRE3 (GCACCAGACAGTGACGTCAGCTGCCAGAT CCCATGGCCGTCATACTGTGACGTCTTTCAGACACCCCATTGACGTCAATGGG AGAAC: SEQ ID No: 9) and CRE5 (TCGAGCCCATGGCCGTCATACTGTGACG TCCCCATGGCCGTCATACTGTGACGTCCCCATGGCCGTCATACTGTGACGTCC CCATGGTCGTCATACTGTGACGTCCCCATGGCCGTCATACTGTGACGTC: SEQ ID No:10).

EXAMPLE 2

Cis-Element/SEAP Constructs

A number of *cis* elements were linked to a secreted alkaline phosphatase reporter wherein the secreted alkaline phosphatase wa used as a reporter. One advantage of using SEAP as a reporter is that SEAP is secreted into the medium and the medium itself can be collected for the assay without disrupting the cells.

In a AP1-SEAP construct, the *cis* element in the construct contains six copies of AP1 binding sequence. In a SRE-SEAP construct, the construct contains three copies of SRE element. In a CRE-SEAP construct, the construct contains three different cAMP responsive elements, responding to cAMP, protein kinase A, forskolin etc. In a GRE-SEAP construct, the construct contains three copies of glucocorticoid responsive element and responds to the glucocorticoid-activated glucocorticoid receptor. In a HRE-SEAP construct, the construct contains three copies of heat responsive element. It responds to a number of types of physiological stress, such as heat. In an NF-κB-SEAP construct, the construct contains four copies of the NF-

κB binding sequence and responds to a number of stimuli, including TNF. In a NFAT-SEAP construct, the construct contains three copies of NFAT binding sequence and responds to PMA, ionomycin, PKC, etc. In a Myc-SEAP construct, the construct contains six copies of myc binding sequence and responds to myc activation. In a P53-SEAP construct, the construct contains two copies of p53 binding sequence, reporting p53 activation.

EXAMPLE 3

pNF-κB-SEAP Construct

pNF-κB-SEAP (SEQ ID No:11) is especially designed for monitoring the activation of NF-κB signal transduction pathway (13-16). The restriction map of pNF-κB-SEAP is shown in Figure 1. Induction of the pathway enables endogenous NF-κB to bind to the kappa (κ) enhancer element (KB_4) located in the promoter region of the vector, thus activating transcription of the reporter gene.

pNF-κB-SEAP allows expression of the secreted alkaline phosphatase reporter gene (17-19). The secreted alkaline phosphatase reporter coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the secreted alkaline phosphatase reporter transcript in eukaryotic cells. A synthetic transcription blocker (TB), composed of adjacent polyadenylation and transcription pause sites located upstream of the κ-enhancer, reduces background transcription (20). The vector backbone also contains an f1 origin for single-stranded DNA production, a pUC origin of replication, and an ampicillin resistance gene for propagation and selection in *E.coli*. The SEAP vectors incorporate a number of additional features that improve the sensitivity of secreted alkaline

phosphatase reporter by increasing the efficiency of secreted alkaline phosphatase reporter expression or that enhance the utility of the vectors. These include: the removal of the SV40 small-t intron, which can cause cryptic splicing and reduced expression in some genes and/or cell types (21-22); switching from the early to late polyadenylation signal of SV40, which typically causes a five-fold increase in mRNA levels (23); compact plasmid size; and removal of extraneous sequence from the 3' untranslated region of the SEAP mRNA.

EXAMPLE 4

Cis-Element/EGFP Construct

EGFP was also used as a reporter to analyze transcription factors. Destabilized EGFP (d2EGFP) is an autofluorescence protein wherein fluorescence emission of this protein does not require any additional substrates or cofactors. d2EGFP is an ideal reporter to be used in cell-based assays for high throughput drug screening as it allows real-time monitoring of activation of transcription factors. The detection can be made in multiple points.

A set of d2EGFP reporters with different cis-elements were generated, which are used for monitoring different transcription factors. Establishment of stable cell lines that express individual reporters expands application of this cis-element reporter in the cell-based high throughput drug screening.

An NF-κB-d2EGFP construct also is used to monitor the activation of NF-κB. This construct responds to a number of stimuli, including TNF and IL1. The AP1-d2EGFP construct is used to monitor the activation of AP1, which responds to growth factors. Additionally, an SRE-d2EGFP construct is used to monitor the activation of ElK1. This

construct responds to serum. The GRE-d2EGFP construct was used to monitor the activation of glucocorticoid receptors and responds to glucocorticoid activation. The CRE-d2EGFP construct was used to monitor CREB activation and responds to cAMP and cAMP-mediated protein kinase A (PKA). An NFAT-d2EGFP construct was used to monitor Ca++-mediated NFAT activation which responds to Ca++. The HSE-d2EGFP construct is used to monitor HSF activation and responds to a stress, such as heat. The P53-d2EGFP construct is used to monitor p53 activation, responding to DNA damage. The Myc-d2EGFP construct is used to monitor Myc activation, which responds to cell growth.

To construct the cis-acting d2EGFP reporters, the binding sequences of *cis* element was fused to the thymidine kinase (TK) promoter and d2EGFP gene. Resulting constructs are able to monitor the activation of a number of transcription factors. In CRE-d2EGFP transfected CHO cells, forskolin treatment elevates intracellular cAMP resulting in induction of d2EGFP. In NFAT-d2EGFP transfected HEK 293 cells, ionomycin and PMA treatment activates NFAT resulting in induction of d2EGFP. In GRE-d2EGFP transfected Saos2 cells, dexamethasone treatment activates glucocorticoid receptor (GR) resulting induction of d2EGFP.

EXAMPLE 5

pNF-κB-d2EGFP Construct

By linking NF-κB binding sequences and the thymidine kinase promoter, d2EGFP gene was able to detect TNF-mediated activation of NF-κB. pNF-κB-d2EGFP (SEQ ID No:12) was designed to monitor the activation of the NF-κB signal transduction pathway (13-16). The restriction map of pNF-κB-d2EGFP is shown in Figure 2.

Induction of the pathway enables endogenous NF-κB to bind to the kappa (κ) enhancer element (KB_4) located in the promoter region of the vector, thus activating transcription of the reporter gene.

d2EGFP is a destabilized variant of the enhanced green fluorescent protein. Unlike the original EGFP protein which is extremely stable, d2EGFP has a half-life of approximately two hours *in vivo* (24). Therefore, d2EGFP provides a more accurate measurement of transient activation by NF-κB than unmodified EGFP and is ideal for kinetic studies of gene activation. To construct d2EGFP, residues 422-461 of mouse ornithine decarboxylase (MODC) were fused to the C-terminus of EGFP. This region of the mouse ornithine decarboxylase contains a PEST amino acid sequence that targets the protein for degradation results in rapid protein turnover. pNF-κB-d2EGFP allows for real-time analysis of pNF-κB activation by either fluorescence microscopy or flow cytometry.

The d2EGFP coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the d2EGFP transcript in eukaryotic cells. A synthetic transcription blocker (TB), composed of adjacent polyadenylation and transcription pause sites located upstream of the κ enhancer reduces background transcription (20). The vector backbone also contains f1 origin for single-stranded DNA production, a pUC origin of replication, and an ampicillin resistance gene for propagation and selection in *E.coli*. The d2EGFP vectors incorporate a number of features that improve the sensitivity of d2EGFP by increasing the efficiency of d2EGFP expression or that enhance the utility of the vectors. These include: the removal of the SV40 small-t intron, which can cause cryptic splicing and reduced expression in some genes and/or cell types (21-22); switching from the early to late polyadenylation signal of SV40, which typically

causes a five-fold increase in mRNA levels (23); compact plasmid size; and removal of extraneous sequence from the 3' untranslated region of the d2EGFP mRNA.

EXAMPLE 6

CRE5-d2EGFP Construct

pCRE5-d2EGFP (SEQ ID No:13) was constructed using the pTK-SEAP as the backbone. The thymidine kinase promoter was substituted by 5 copies of CREs (cAMP response elements) fused with gonadotropin α-gene promoter (also called Thyroid-stimulating hormone TSHα subunit). The d2EGFP reporter gene was used instead of secreted alkaline phosphatase. This construct was designed to monitor the cAMP level changes *in vivo*. cAMP elevation within cells upon stimulation should result in the phosphorylation of CREB, which consequenly induces the transcription and translation of the reporter gene d2EGFP. The destabilized form of EGFP eliminated the accumulation of EGFP in cells and better represented the changes of its expression level upon stimulation.

The stable cell line was generated by stably transfected pCRE5-d2EGFP together with pSV2-neo into CHO-K1 cells. Transfected cells were selected under G418 at a 500 μg/ml concentration and individual clones were isolated and analyzed for forskolin induction. Three cell clones were selected which show a time course as well as the positive response to forskolin. Clone CRE5-dE #3 has the highest fluorescence intensity and is the easiest to detect induction, since it had five fold increase of fluorescence after induction. Clone CRE5-dE #38 has the highest fold of induction (10 fold) and the lowest background EGFP expression. However, after induction, Clone CRE5-dE

#38 had a lower fluorescent intensity than that of clone #3. Clone #38-2 is an intermediate of above two clones, having an 8 fold fluorescence induction and, after induction, the fluorescence intensity was at the similar level of clone #3. These cell clones can be used in cell based high-throughput screening in search of factors involved in cAMP signal transduction pathway.

EXAMPLE 7

Cis-Element/Luciferase Construct

Luciferase was also used as a reporter to analyze transcription factors. Luciferase is one of most sensitive reporters and is widely used in the art. Due to its sensitivity and popularity, luciferase was selected to link to a *cis* element to form a *cis*-element/luciferase construct for monitoring the activation of transcription factors.

pNF-κB-Luciferase (pNF-κB-Luc, SEQ ID No:14) contains a firefly luciferase gene and is designed for monitoring the activation of the NF-κB signal transduction pathway (13-16). The restriction map of pNF-κB-Luc is shown in Figure 3. Induction of the pathway enables endogenous NF-κB to bind to the kappa (κ) enhancer element (KB_4) located in the promoter region of the vector, thus activating transcription of the reporter gene. The luciferase coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the luciferase transcript in eukaryotic cells. A synthetic transcription blocker, composed of adjacent polyadenylation and transcription pause sites located upstream of the κ-enhancer, reduces background transcription (20). The vector backbone also contains an f1 origin for single-stranded DNA production, a pUC origin

of replication, and an ampicillin resistance gene for propagation and selection in *E.coli*.

The pNF-κB vectors incorporate a number of features that improve the sensitivity of luciferase by increasing the efficiency of luciferase expression or that enhance the utility of the vectors. These include: the removal of the SV40 small-t intron, which can cause cryptic splicing and reduced expression in some genes and/or cell types (21-22); switching from the early to late polyadenylation signal of SV40, which typically causes a five-fold increase in mRNA levels (23); compact plasmid size; and removal of extraneous sequence from the 3' untranslated region of the luciferase mRNA.

EXAMPLE 8

Application of *Cis*-Acting Reporters in Assaying the Activity of Transcription Factors in Signal Pathways

The activity assay of the present invention may be carried out in a 12 or 24 well plate. This assay can be applied to a lot of studies, such as examining whether a new target gene links to any signal pathways. The assay can be used for establishing a functional status profile of transcription factors for evaluating any biological difference *in vivo*, such as normal cells *versus* differentiated, apoptotic, and cancer cells. The assays of the present invention can also be used to examine and quickly identify biological markers for human diseases. Drug validation is another application of the present assay.

Figure 4 demonstrates mediated induction of transcription factors in HEK 293 cells. Figure 4A shows tumor necrosis factor (TNF)-mediated transcriptional induction. Human embryonic kidney (HEK 293) cells were transfected individually with each reporter vector and

incubated for 24 hr in serum-free media. After incubation with TNF for 6 hr, samples were collected and measured for SEAP activity. The transcriptional profile reveals that, as expected, TNF activated the NFκB and the NFAT signaling pathways, which are known to play pivotal roles in the transcription of genes involved in the immune response (1-4). Other transcription factors and controls were unaffected by TNF stimulation.

Figure 4B shows serum-mediated induction of transcription factors HEK293 cells. After transfection, cells were cultured in serum-free media for 24 hr and incubated with 10% serum for 6 hr. Samples were collected and measured for SEAP activity as described above. Unlike the TNF-mediated activation of specific pathways (Figure 4A), serum activates multiple transcription factors that induce a variety of signaling events after stimulation.

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Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A construct for measuring transcription, comprising:

a *cis* element, wherein said *cis* element is selected from the group consisting of nuclear factor-κB having the sequence shown in SEQ ID No: 1, heat responsive element having the sequence shown in SEQ ID No: 2, Myc having the sequence shown in SEQ ID No: 3, p53 having the sequence shown in SEQ ID No: 4, nuclear factor of activated T cells having the sequence shown in SEQ ID No: 5, alkaline phosphatase 1 having the sequence shown in SEQ ID No: 6, serum responsive element having the sequence shown in SEQ ID No: 7, glucocorticoid responsive element having the sequence shown in SEQ ID No: 8 and cAMP responsive element having the sequence selected from the group consisting of SEQ ID No: 9 and SEQ ID No: 10;

a reporter gene; and

a promoter.

2. The construct of claim 1, wherein said reporter gene is selected from the group consisting of secreted alkaline phosphatase, destabilized green fluorescent protein and luciferase.

3. The construct of claim 1, wherein said promoter is selected from the group consisting of thymidine kinase promoter and gonadotropin α-gene promoter.

4. A vector comprising the construct of claim 1.

5. The vector of claim 4, wherein said vector is pNF-κB-SEAP.

6. The vector of claim 4, wherein said vector is pNF-κB-d2EGFP.

7. The vector of claim 4, wherein said vector is pNF-κB-Luc.

8. A method of monitoring activation of a transcription factor, comprising the steps of:

generating the construct of claim 1, wherein said *cis*-element is a transcription factor;

transforming a cell line with a vector containing said construct; and

detecting the expression of said reporter gene, wherein expression of said reporter gene indicates the activation of said transcription factor.

9. A method of monitoring activation of a transcription factor, comprising the steps of:

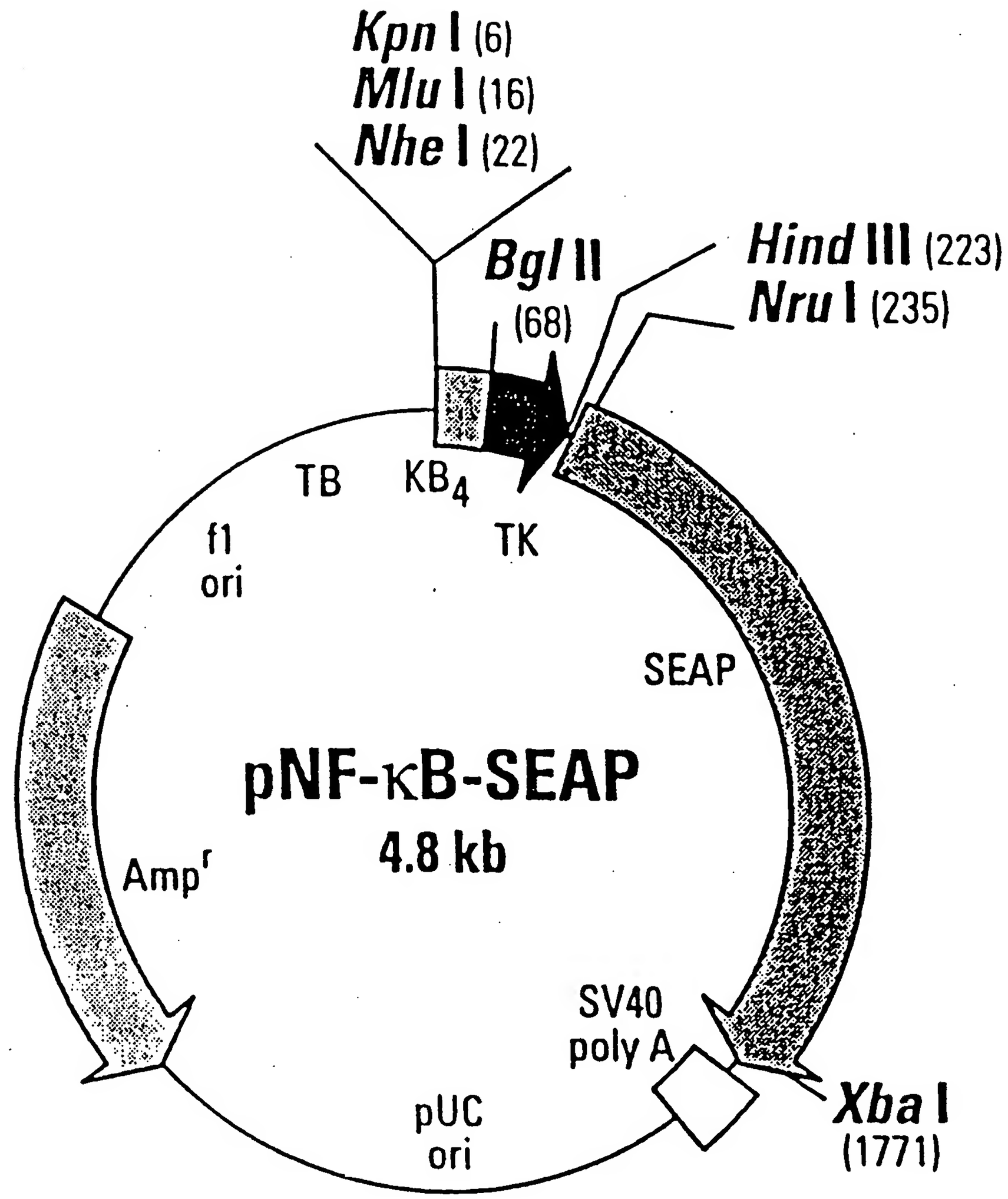
generating the construct of claim 2;

transforming a cell line with a vector containing said construct; and

detecting the expression of said reporter gene, wherein expression of said reporter gene indicates the activation of said transcription factor.

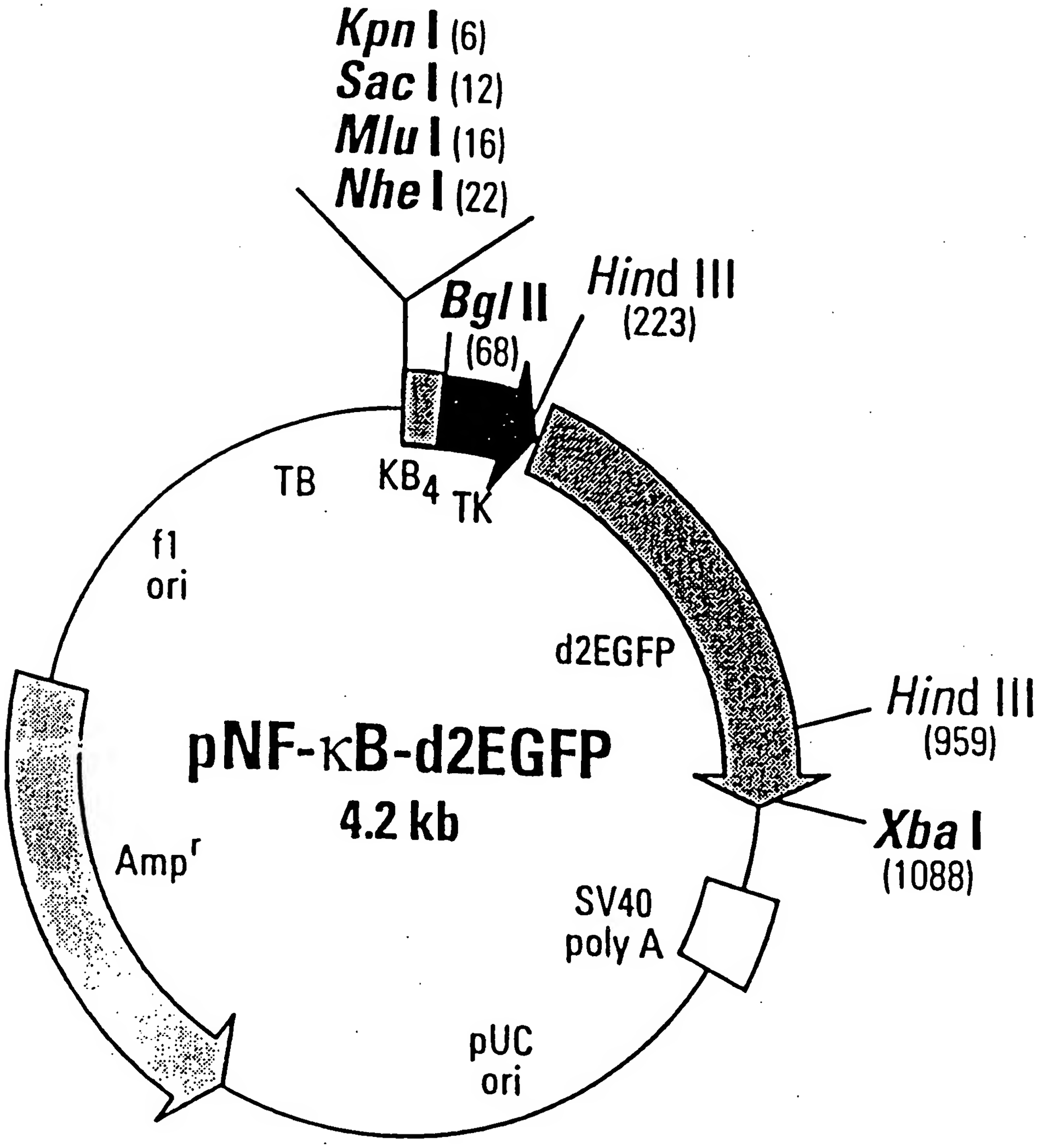
10. The method of claim 9, wherein said cell line is selected from the group consisting of CHO, HEK 293 and Saos2.

11. The method of claim 9, wherein said construct is selected from the group consisting of pNF-κB-SEAP, pNF-κB-d2EGFP and pNF-κB-Luc.



TB = Transcription Blocker

FIG. 1



TB = Transcription Blocker

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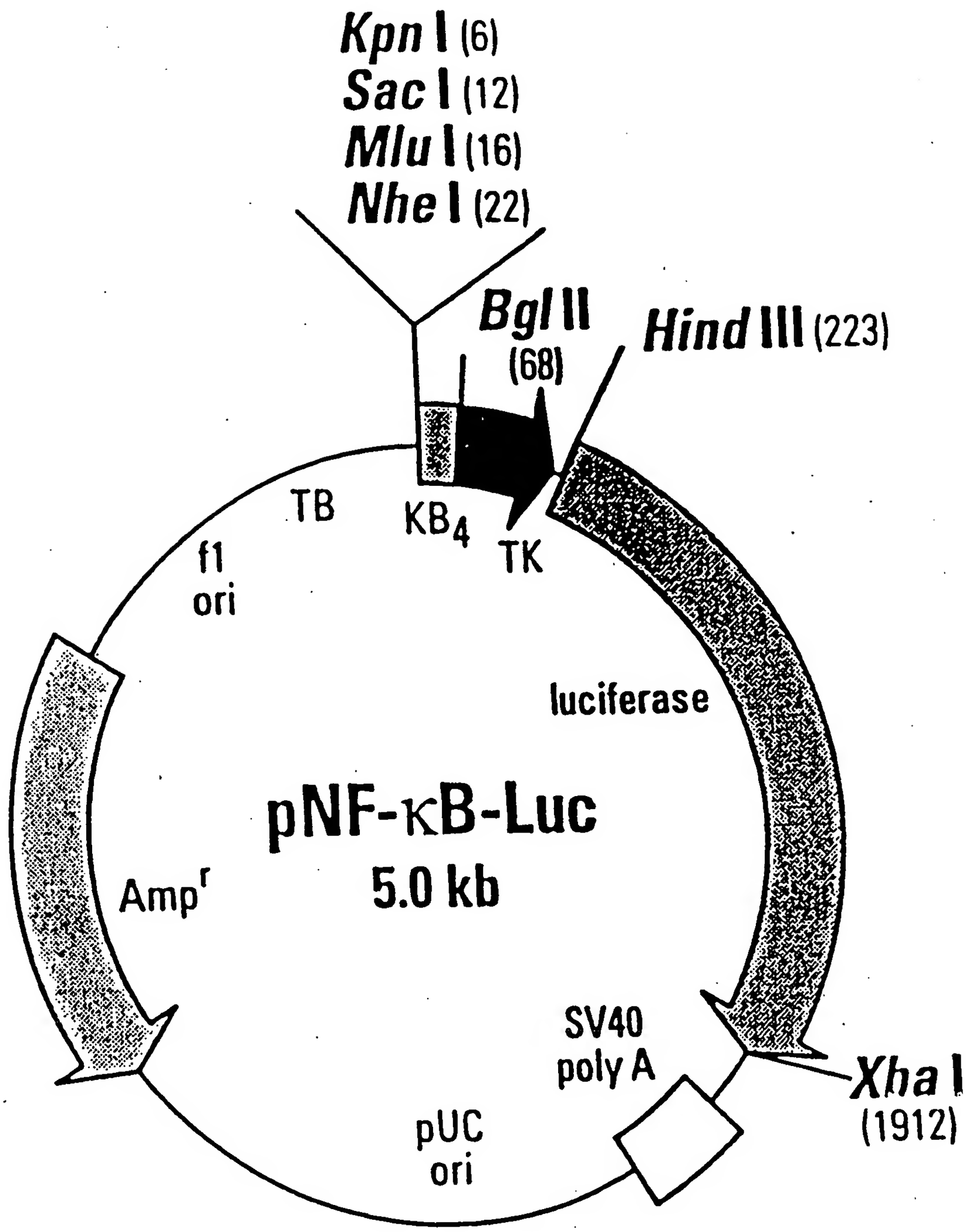

TB = Transcription Blocker

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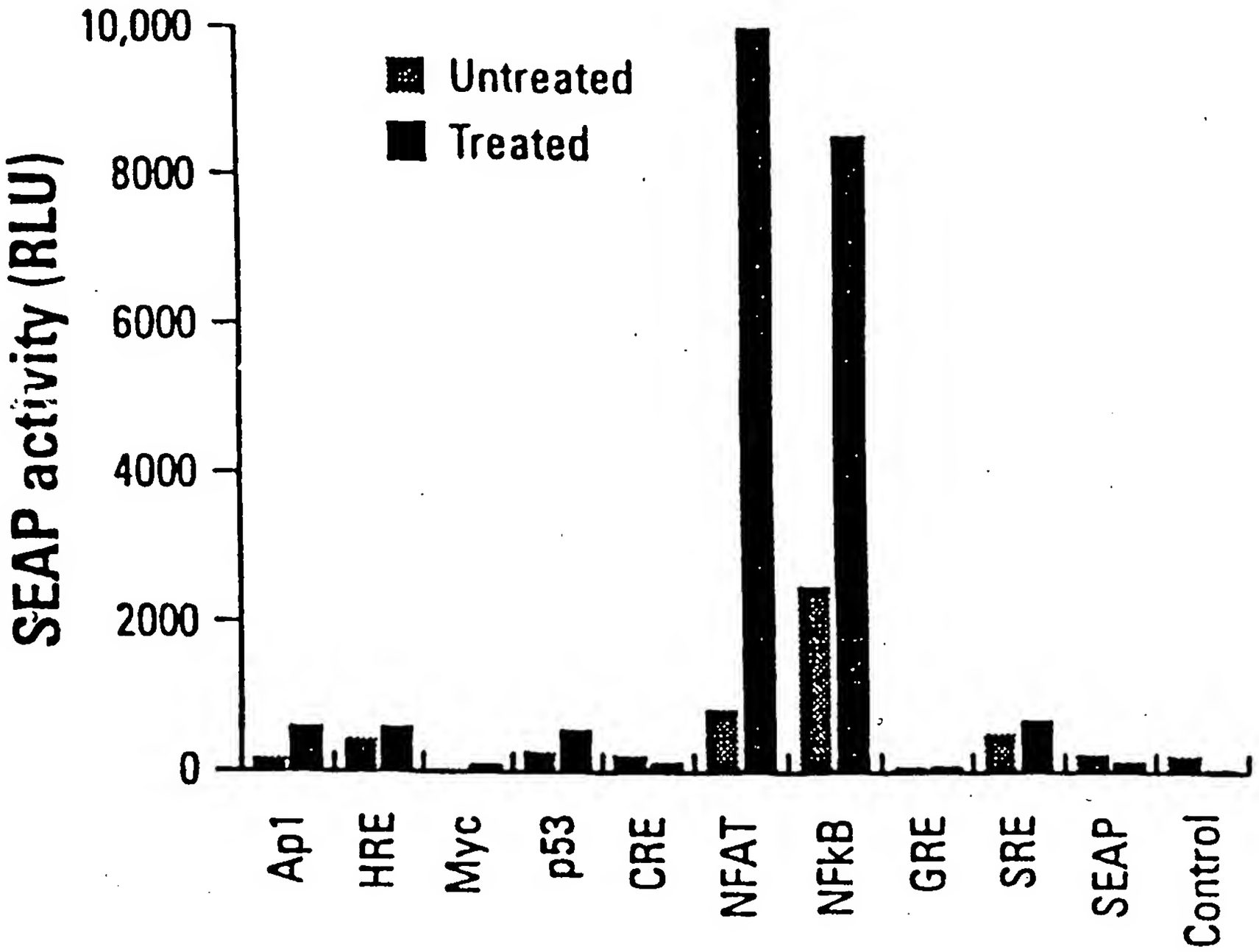
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FIG. 4A

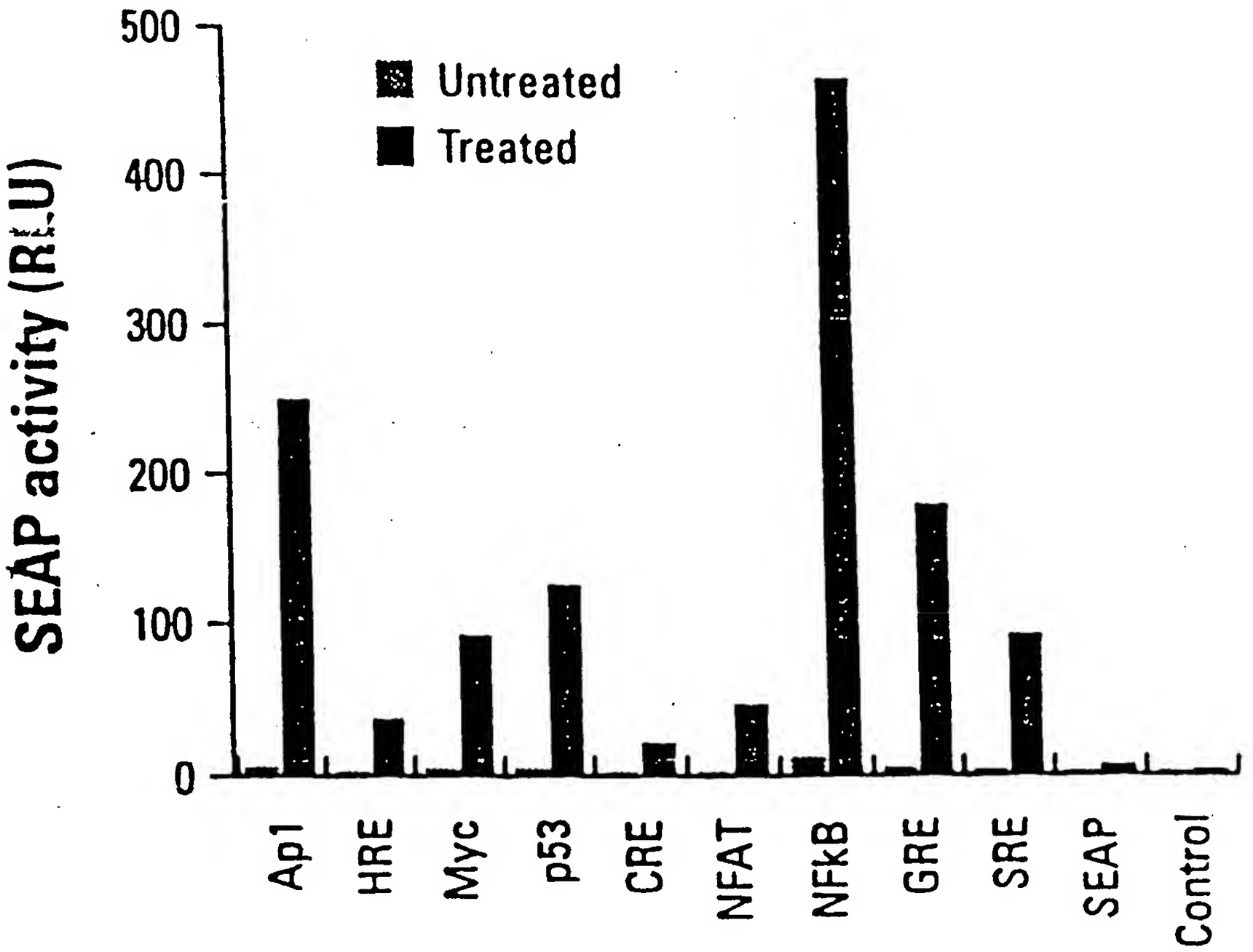
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FIG. 4B

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SEQ 14/14